PREDICTING GENTAMICIN INDUCED NEPHROTOXICITY USING PHARMACOMETABONOMIC APPROACH

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by

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LIST OF ABBREVIATIONS

AECUSM	Animal Ethics Committee USM
AKI	Acute kidney injury
AKIN	Acute kidney injury network
AMDI	Advanced Medical and Dental Institute
AMIX	Analysis of mixture
AP	Alkaline Phosphatase
AQ	Acquisition time
ASA PS	American Society of Anaesthesiologists Physical Status
ATM	Automatic tuning and matching
ATN	Acute tubular necrosis
AUC	Area under curve
AUROC	Area under receiver operating characteristics
B-BIOREFCODE	Bruker Biofluid Reference Compound Database
BBO	Broadband observe
BMRB	Biological Magnetic Resonance Data Bank

BUN	Blood urea nitrogen
Carb Hb	Carbamylated Hb
CHF	Congestive heart failure
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
COSY	Correlation spectroscopy
CPMG	Carr-Purcell-Meiboom-Gill
CRC	Clinical Research Centre
CrCl	Creatinine clearance
CRRT	Continuous renal replacement therapy
D1	Delay parameter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DS	Number of dummy scans
DSA	4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate
DSS	2,2-dimethyl-2-silapentane-5-sulfonic acid
eCrCl	Estimated creatinine clearance

EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
FID	Free induction decay
FSGS	Focal segmental glomerulosclerosis
FT	Fourier transform
G6P	Glucose-6-phosphate
GC-MS	Gas chromatography-mass spectrometer
GC-TOF	Gas chromatography-time-of-flight
GFR	Glomerular filtration rate
ɤ-GST	ɤ-gluthathione-S-transferase
GGT	ɤ-glutamyltransferase
GST	Gluthathione-S-transferase
H&E	Haematoxylin and eosin
HDL	High density lipoprotein
HMDB	Human Metabolome Database
HSQC	Heteronuclear single quantum coherence

ICU	Intensive care unit
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-6	Interleukin-6
IL-8	Interleukin-8
IRRT	Intermittent renal replacement therapy
IQR	Interquartile range
IV	Intravenous
kDa	Kilo dalton
KDIGO	Kidney Disease Improving Global Outcomes
KIM-1	Kidney injury molecule-1
LC	Liquid chromatography
LCECA	Liquid chromatography electrochemical array
LC-MS	Liquid chromatography- mass spectrometer
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
MC	Mean centring

MCD	Minimal change disease
MDRD	Modification of Diet in Renal Disease
MIC	Minimum inhibitory concentration
MMP-9	Matrix metalloproteinase-9
MOH	Ministry of Health
MREC	Medical Research and Ethics committee
MS	Mass spectrometer
NADH	Nicotinamide adenine dinucleotide
NAG	N-acetyl-β-ɒ-glucosamidase
NGAL	Neutrophil gelatinase-associated lipocalin
NHE3	Sodium-hydrogen exchanger isoform-3
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
NS	Number of scan
NSAIDs	Nonsteroidal anti-inflammatory drugs
O1p	Centre of spectra
OD	Once daily

OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
p1	Pulse length
PAR	Pareto
PAS	Periodic-acid Schiff
PCA	Principal Component analysis
PLS	Partial Least Squares
PLS-DA	Partial Least Squares Discriminant Analysis
Pro-ANP	Prohormone of atrial natriuretic peptide
Q^2	Goodness of prediction
QSAR	Quantitative Structure Activity relationship
R^2	Goodness of fit
RBP	Retinol-binding protein
RCF	Relative centrifugal force
RG	Receiver gain
RIFLE	Risk, injury, failure, loss and end stage kidney disease
ROA	Route of administration
ROS	Reactive oxygen species

RRT	Renal replacement therapy
SC	Subcutaneous
SCr	Serum creatinine
SD	Sprague Dawley
SD	Standard deviation
SIMCA	Soft Independent Modelling of Class Analogy software
SPSS	Statistical Package for the Social Sciences
SWH	Sweep width in hertz
TD	Time domain
TMNO	Trimethylamine-N-Oxide.
TMOA	Trimethylamine-N-oxide
TMS	Tetramethylsilane
TSP	Sodium salt of 2,2,2,2,-tetradeutero-4,4- dimethyl-4-silapentanoic acid
UO	urine output
UV	Unit variance
Vd	Volume distribution
VIP	Variable Importance in Projection

α-GST	α-gluthathione-S-transferase
π-GST	π-gluthathione-S-transferase

LIST OF SYMBOLS

1D	One dimensional
2D	Two dimensional
Cl^-	Chloride
dl	deciliter
D_2O	Deuterated water
H^+	Hydrogen
H_2O	Water
Hz	Hertz
K^+	Potassium
$K_2\ HPO_4$	Dipotassium phosphate
m/z	mass over charge
mg	miligram
MHz	mega hertz
$Na\ H_2PO_4$	Monosodium phoshate
Na^+	sodium
$Na_2\ HPO_4$	Disodium phosphate

NaF	Sodium flouride
NaN_3	Sodium azide
ppm	part per million
δ	Chemical shift

MERAMAL KENEFROTOKSIKAN ARUHAN GENTAMICIN MENGGUNAKAN PENDEKATAN PHARMACOMETABONOMIC

ABSTRAK

Gentamicin merupakan antibiotik yang digunakan secara meluas namun kesan kenefrotoksikan menjadi kebimbangan utama kerana ia disaring melalui buah pinggang untuk disingkirkan ke dalam urin. Walaubagaimanapun, diagnosis pada peringkat awal adalah sukar dan tiada metabolit yang boleh digunapakai sebagai penanda bio yang tersedia. Maka, dalam kajian ini, penyelidikan terhadap perubahan pola metabolit di dalam sampel serum dan urin sebelum administrasi gentamicin dijalankan melalui pendekatan 'Pharmacometabonomic' menggunakan NMR dan model untuk meramal kenefrotoksikan aruhan gentamicin dibangunkan seterusnya mengenalpasti metabolit berkaitan sebagai penanda bio. Matlamat kajian ini adalah menentukan potensi metabolit di dalam urin dan serum untuk meramal kenefrotoksikan aruhan gentamicin. Seterusnya, model pre-dos dibangunkan menggunakan sampel serum dan urin dalam tikus 'Sprague Dawley' (SD) yang di selidik menggunakan spektroskopi NMR bersama analisis statistik multivariate seperti analisis komponen utama (PCA) dan analisis orthogonal separa persegi terdiskriminasi (OPLS-DA); kemudian, satu set metabolit sebagai penanda bio dikenalpasti. Seterusnya, model yang telah dibangunkan disahkan di dalam sekumpulan tikus SD dan manusia. Gentamicin menghasilkan kesan toksik pada

sebahagian tikus SD, tetapi memberi kesan yang sedikit pada yang lain (kumpulan tidak toksik), seperti yang dinilai melalui keputusan kimia klinikal untuk kenefrotoksikan dan histologi. Selanjutnya, analisis statistik multivariat menunjukkan pemisahan yang signifikan di antara tikus SD yang toksik dengan tidak toksik dalam model yang telah dibangunkan. Model pre-dos serum dicirikan oleh satu set metabolit yang terdiri daripada laktat, trimethylemin-N-oksida, xilosa, deoxyinosine, 2-fosfogliserat and glukosa-6-fosfat. Manakala model pre-dos urin, dicirikan oleh satu set metabolit seperti asid 4-pyridoxic, 2-oxoglutarate, sitrat, betaina, asid hipurik, alantoin and urea. Metabolit ini dikesan melalui serum dan urin di dalam tikus SD yang mengalami nefrotoksik sebelum administrasi gentamicin dan berhubungkait dengan glikolisis anaerobik, kegagalan metabolism purina dan hiperuremia, kitaran asid trikarbosilik dan penekanan urin asid 4-piridoksik. Selain itu, model pre-dos serum dan urin juga telah divalidasi dalam tikus SD dan manusia namun keputusan kesensitifan, kejituan dan ketepatan model adalah rendah disebabkan saiz sampel yang kecil. Dapatan ini membuktikan model yang dihasilkan melalui pendekatan ‘pharmacometabonomic’ berpotensi untuk meramal kenefrotoksikan aruhan gentamicin sebelum pemberian dos gentamicin dan untuk pemahaman lanjut tentang ciri setiap metabolit yang dikenalpasti.

PREDICTING GENTAMICIN INDUCED NEPHROTOXICITY USING PHARMACOMETABONOMIC APPROACH

ABSTRACT

Gentamicin has been one of the most widely used antibiotics. However, its nephrotoxicity is a major concern as it is filtered through the kidneys for excretion in the form of urine. However, early diagnosis is difficult and no reliable metabolites as biomarkers are currently available. Thus, in this study, a pharmacometabonomic approach using nuclear magnetic resonance (NMR) spectroscopy to investigate the altered metabolic pattern in serum and urine samples prior to administration of gentamicin has been employed to develop a model to predict nephrotoxicity induced by gentamicin and to identify the metabolic biomarkers associated. The aim of this study is therefore to determine the potential of urine and serum metabolites in predicting gentamicin induced nephrotoxicity. Subsequent to this, a pre-dose model was developed using urine and serum samples in a number of Sprague Dawley (SD) rats under investigation using NMR spectroscopy. The finding were subjected, first to a multivariate statistical analysis, such as a principal components analysis and orthogonal partial least-squares discriminant analysis; then, a set of metabolites biomarkers was identified. Next, the developed models were validated in groups of SD rats and human subjects. Gentamicin produced toxic responses in some SD rats (toxic group), but had little effect in others (nontoxic group), as judged by their clinical chemistry for nephrotoxicity and histological results. The multivariate statistical analysis following this showed a significant separation between toxic and

non-toxic SD rats, from which the model was developed. The pre-dose serum was characterised by a set of metabolites of lactate, trimethylamine-N-oxide, xylose, deoxyinosine, 2-phosphoglycerate and glucose-6-phosphate. Meanwhile, in the pre-dose urine of the SD rats, a set of metabolites was characterised by 4-pyridoxic acid, 2-oxoglutarate, citrate, betaine, hippuric acid, allantoin and urea of citrate, hippurate and glycine. These metabolites were detected from the serum and urine of SD rats with nephrotoxicity prior to gentamicin administration and associated with anaerobic glycolysis, impaired purine metabolism and hyperuricemia, tricarboxylic acid cycle, and depressed urinary 4-pyridoxic acid. Following this, the pre-serum and urine models were validated in SD rats and human subjects with poor sensitivity, specificity and accuracy due to a limitation in sample size. These findings demonstrate that the models produced by the pharmacometabonomic approach may be useful in predicting gentamicin induced nephrotoxicity prior doses and in arriving at a deeper understanding of the characteristics of each metabolite identified.

CHAPTER 1 – INTRODUCTION

Gentamicin is a well known antibiotic causing kidney injury in proximal tubules is a very crucial matter. Kidney is a vital organ in human body. These pair of bean shaped organ important function is in execratory of waste produced by human body. Other than that, kidney also important in regulation of blood pressure, minerals, red blood cells production and blood acid-base balance (Rylander et al., 2006). Importantly, drug induced nephrotoxicity contribute about 19-25% of kidney injury (Avent et al., 2011; Begg & Barclay, 1995). Nephrotoxicity is a term given for any poisonous or insult to the kidney. Many studies have been conducted worldwide to overcome and improve drug induced nephrotoxicity incidence in human. The techniques used for diagnosing and monitoring nephrotoxicity are evolving continually with new methods emerging including the discovery of biomarkers. This includes the new omics technology such as metabolomic, metabonomics and pharmacometabonomic. This new method had a promising hope in drug treatment which tailored to patients characteristics, thus it will improve efficacy and reduce the number and severity of nephrotoxicity. The objective of current study is to experiment, determine and evaluate the potential of urine and blood metabolite in predicting gentamicin induced nephrotoxicity. The role of this study to help the clinician personalized drug treatment according to patients need which it can maximize the benefits of the treatment and at the same time minimize the side effects.

1.1 Limitations of Current Practice

No proven methods are available to predict gentamicin induced nephrotoxicity prior to administration. Until the development of new biomarkers, detection of nephrotoxicity will only be discovered after the administration of gentamicin and/or until an overt change in renal function.

1.2 Statement of the Problem

To-date, no studies/methods have been proven to predict gentamicin induced nephrotoxicity in individuals prior to administration of the drug. In general, the detection of nephrotoxicity will only be perceived after administration of antibiotics/drugs and/or until an overt change in renal function is evident. In the current practice of hospitals, gentamicin is monitored using drug concentration in the blood after initiation of therapy. The present study, hopefully, can address current limitations. The objective of the current study is therefore to experiment, determine and evaluate the potential of human urine and serum metabolites in predicting antibiotic induced nephrotoxicity. It aims to help the clinician personalise drug treatment according to the patient's need in order to maximise the benefits of the treatment and at the same time minimise its side effects.

1.3 Study Hypothesis

This study hypothesises that gentamicin induced nephrotoxicity can be predicted using a pre-dose metabolic profile of urine and serum via pharmacometabonomic method using NMR.

1.4 Study Objectives

1.4.1 General Objective

The objectives of the current study are to determine the potential of urine and serum metabolites in predicting gentamicin induced nephrotoxicity.

1.4.2 Specific Objective(s)

The specific objectives of the study were to:

1. Explore the potential of pre-dose urine and serum metabolites to discriminate between subjects with and without gentamicin toxicity
2. Observe changes in metabolites of the urine and serum of subjectsbefore and after treatment with gentamicin
3. Associate the identified metabolites with the relevant clinical chemistry and histopathology of the kidney
4. Determine the putative metabolites that could predict gentamicin induced nephrotoxicity
5. Evaluate the possibility of using the same metabolites in predicting gentamicin induced nephrotoxicity in rats and humans.

CHAPTER 2 – LITERATURE REVIEW

2 The Kidney

The kidneys, also known as renals, are a vital organ in the human body. This pair of bean-shaped organs, each about the size of a clenched fist, and the adrenal glands sitting on top of them, release renin which affects blood pressure as well as sodium and water retention. The primary function of the kidneys is in the excretion of waste produced by the body and the removal of excessive fluid. In addition to that, the kidneys are also important in the regulation of blood pressure, minerals, production of red blood cells and maintaining the blood acid-base balance (Lattanzio & Kopyt, 2009).

The kidneys are found along the posterior muscular wall of the abdominal cavity. They lie behind the peritoneum that lines the abdominal cavity and are thus considered to be retroperitoneal organs. They are protected from external damage by the ribs and muscles of the back. Additionally, the adipose tissue known as perirenal fat which surrounds the kidneys acts as protective padding. If each kidney were bisected from top to bottom, the two major regions of the granular cortex and medulla could be visualised. Nephron is the functional unit of the kidneys which makes up one million functional units in the kidneys. Nephron has two main parts: one is the glomerulus. The other is Bowman's Capsule found in the renal corpuscle and renal tubule, consisting of a proximal convoluted tubule, loop of Henle, a distal convoluted tubule, a collecting tubule and a collecting duct (Figure 2.1) (Deshmukh & Wong, 2009).

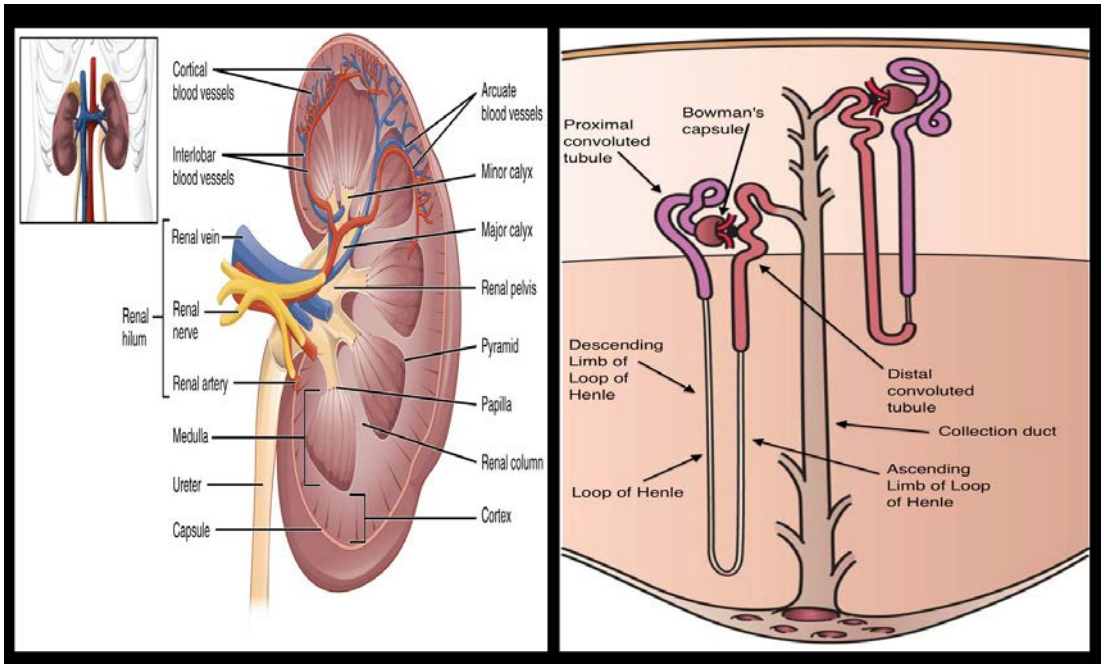

Figure 2.1: Human Kidney Anatomy. Adapted from (Deshmukh & Wong, 2009)

Nephron is responsible for filtering the blood in the kidneys according to three main processes known as glomerular filtration, tubule secretion and tubule reabsorption. Blood enters the glomerulus via afferent arterioles under high pressure in the renal corpuscles, forcing substances to be sieved across the leaky endothelial-capsular membrane and podocyte into the nephron, passing Bowman's capsule into proximal convoluted tubules. Podocytes, which are special epithelial cells formed from the layer of the Bowman's capsule surrounding the capillaries of the glomerulus, work with the endothelium of the capillaries to separate the urine from blood passing through the glomerulus into the kidney tubules. An ultra-filtration process transports to the kidney tubules substances consisting of water, small proteins, salts (Na^+, Cl^-, K^+, H^+), glucose, nitrogenous waste products, such as urea, and other metabolic waste together with drug metabolite residues in the plasma and protein in the blood.

Tubular reabsorption begins as soon as the filtrate enters the proximal convoluted tubule as the most active but selective reabsorption agent. This process involves near total reabsorption of organic nutrients, and the hormonally regulated reabsorption of water and ions. Sodium, bicarbonate, glucose, amino acids, vitamins, and most cations enable ion reabsorption via active transportation, whilst other substances such as water, lipid soluble solutes and urea reabsorbed via passive diffusion driven by chemical or concentration gradient. The proximal tubule is also an important site for the secretion of organic acids and bases such as bile salts, oxalate, urate and catecholamines (Christensen & Birn, 2001).

The descending limb of the loop of Henle is permeable to water and reabsorbs via osmosis. The ascending limb, however, though impermeable to water is permeable to electrolytes, which cause reabsorption of sodium, chloride, potassium through active transportation and passive diffusion of magnesium and calcium ions driven by electrochemical gradient and hydrogen ions secreted into the lumen. The first part of the distal tubule forms part of the juxtaglomerular complex that provides feedback control of the glomerular filtration rate and blood flow, and the continuing distal tubule segment absorbs most electrolytes in the same way as the ascending loop of Henle. On the other hand, the late distal tubule and cortical collecting tubule reabsorb sodium, potassium ions and water, while secreting hydrogen ions into the tubular lumen. The final urine output depends on the absoption of water and sodium in the collecting duct which depends on its antidiuresis hormoneal level (Deshmukh & Wong, 2009). Figure 2.2 summarize activities involve in kidney.

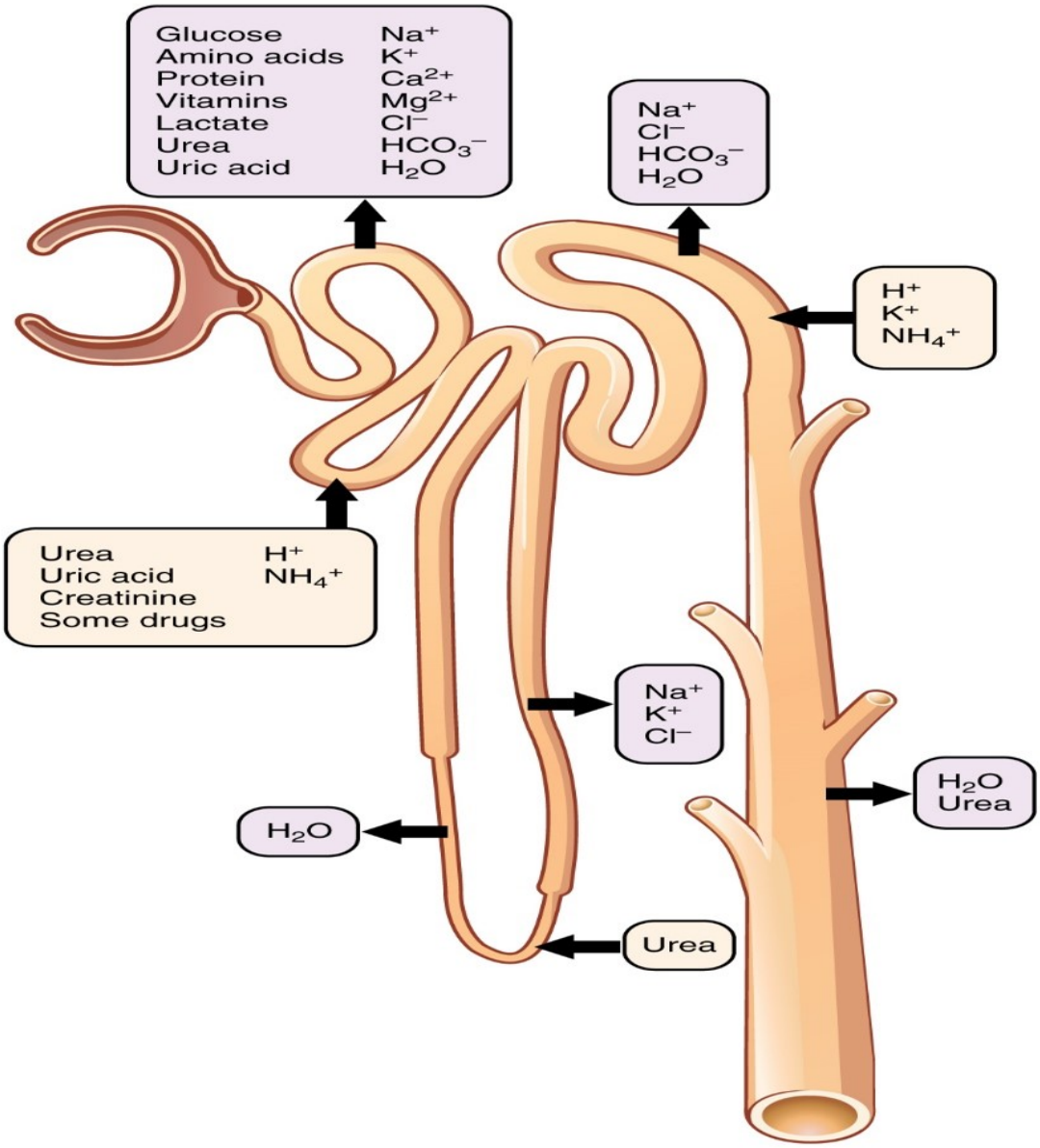

Figure 2.2: Secretion and reabsorption of various substances throughout the nephron in kidney. Adapted from (Deshmukh & Wong, 2009)

2.1 Acute Kidney Injury

2.1.1 Epidemiology

The true overall epidemiology of AKI is limited because of the different definitions of AKI and the variation in populations around the world. AKI is known in layman's terms as sudden impairment of kidney function. Although many definitions of AKI have being published, the Clinical Practice Guidelines produced from Kidney Disease Improving Global Outcome (KDIGO) and the UK Renal Association have agreed to define AKI as shown in Table 1.1 (Association, 2007; Khwaja, 2012b).

Table 2.1: Definition of AKI

AKI is defined by the criteria below:
• Increase in serum creatinine (SCr) by x0.3 mg/dl (x 26.5 µmol/l) within 48 hours; or • Increase in SCr to x1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or • Urine volume 0.5 ml/kg/h over 6 hours

The cause of AKI can be divided into three categories as follows (Choudhury & Ahmed, 2006); prerenal, intrinsic and postrenal AKI. Prerenal AKI resulting from decreased renal perfusion, often as a consequence of cardiac failure, volume depletion and low blood pressure, such as after severe hemorrhage sepsis burns. Intrinsic AKI resulting from abnormalities within the kidney itself, which can be further divided into sub categories (a) AKI caused by glomerularnephritis, (b) AKI caused by tubular necrosis, which might be contributed by severe renal ischemia or toxins and medication. Meanwhile, postrenal AKI, meaning the obstruction of the

urinary system any where from the calices to bladder outflow. Most of the obstructions are caused by abnormalities of the lower urinary tract outside the kidney such as kidney bladder outlet obstruction and kidney stones in both ureters. Nevertheless, some studies have documented the frequent occerrence of AKI in intensive care unit (ICU) cases which possibly affect about two-thirds of such patients (Dennen et al., 2010; Lameire et al., 2008; Parazella, 2010; Uchino, 2006). It is therefore crucial to know the incidence, etiology and clinical features of AKI to promote preventive strategies and to implement adequate resources for the management of AKI. The mortality rate of patients having AKI is approximately 50% and the figure rises to 70% of patients treated in the ICU (Liano et al., 1996; Lins et al., 2000). Almost 55% of the AKI was acute tubular necrosis, of which 15% was caused by medical toxins, while some studies have shown up to 25% (Liano et al., 1996; Lins et al., 2000; Parazella, 2010).

2.1.2 Drug Induced Nephrotoxicity

The incidence of drug induced nephrotoxicity is difficult to determine but about 18.3% cases of acute tubular necrosis (ATN) are caused by medication (Choudhury & Ahmed, 1997). It is notworthy that the reported incidence of nephrotoxicity due to administration of antibiotics is 36% (Choudhury & Ahmed, 2006). Different drugs may cause injury at different sites in the kidneys and such injury caused by drugs can be categorised as tubular damage, glomerular damage, interstitial damage and vascular damage (Choudhury & Ahmed, 2006). The tubules are more susceptible to toxic injury for the following reasons. First, high concentrations of filtered toxicants are present in the tubular fluid due to their function in the reabsorption of solutes and

water. Second, tubular epithelial cells have a large number of transporters, resulting in high intracellular concentrations of toxicants (Choudhury & Ahmed, 1997, 2006; Naughton, 2008). Third, tubular epithelial cells have high energy requirements for supporting metabolism as well as for transporting solutes. Tubular injury can be the result of direct toxicity in the tubular epithelium, or obstructive insult or inflammation. The most frequent drugs leading to tubular injury are aminoglycosides, radiocontrast media, cisplatin, amphothericin B, pentamidine, tacrolimus, cephaloridine, and many more. In glomerular damage, the drugs involved include lithium, pamidronate, sirolimus, NSAIDs and interferons, which lead to conditions known as minimal change disease (MCD) and Focal segmental glomerulosclerosis (FSGS). Most common forms of interstitial damage are acute and happen as a result of allergic reactions that elicit a strong inflammatory response. Antibiotics such as penicillins, cephalosporins, sulfanamides, rifampicin, nonsteroidal anti-inflammatory drugs (NSAIDs), phenytoin and thiazide diuretic have been associated with interstitial damage. The less common form of renal injury caused by drugs are vascular damage, associated with drugs such as propylthiouracil, hydralazine, methimazole, sulfasalazine, phenythoin and minocycline (Begg & Barclay, 1995; Choudhury & Ahmed, 1997, 2006; Parazella, 2010). Other than that, acute and chronic risk factors as listed in Table 2.1 might increase the risk of drug induced nephrotoxicity.

Table 2.2: Risk factors for drug induced nephrotoxicity (Parazella, 2010)

Chronic risk factors	Acute risk factors
Age > 65 years	Sepsis/infection
Chronic kidney disease	Volume Depletion
Diabetes mellitus	Acute decompensated heart failure
Malignancy	Hypotension
Cardiovascular disease	Complex & major surgery
Peripheral vascular disease	Trauma
	Mechanical ventilation

2.2 Aminoglycosides

Aminoglycoside antibiotics, which are derived from *Streptomyces* and *Micromonospora*, have very potent bactericidal activity (Avent et al., 2011). The basic chemical structure required for both potency and the spectrum of antimicrobial activity of aminoglycosides is that of one or several aminated sugars joined in glycosidic linkages to a dibasic cyclitol Figure 2.3 (Mingeot-leclercq & Tulkens, 1999). Currently, there are nine (9) aminoglycosides available in the market: gentamicin, tobramycin, amikacin, streptomycin, neomycin, kanamycin, paromomycin, netilmicin and spectinomycin as listed in Table 2.3 and the chemical structure as shown in Figure 2.3.

Gentamicin, tobramycin and amikacin are the most widely prescribed by physicians (Radigan et al., 2010). Most aminoglycosides are admistered via the parentral route as they are not absorbed through the stomach (Radigan et al., 2010; Tulkens et al., 1999). A significant clinical toxicities, such as nephrotoxicity and ototoxicity, can result from aminoglycosides. Clinical studies have shown that about 5-25% of adult patients receiving aminoglycosides experienced drug induced toxicity (Avent et al., 2011; Choudhury & Ahmed, 1997; Guo & Nzerue, 2002; Hock & Anderson, 1995; Meyer, 1986) (Pannu & Nadim, 2008) (Radigan et al., 2010).

2.2.1 Gentamicin

Gentamicin is one of the most widely prescribed drugs in the aminoglycoside group. Gentamicin is effective for serious infections caused by Gram-negative organisms (Avent et al., 2011; Leong et al., 2006; Selby et al., 2009). Rapid bactericidal activity and comparatively low incidence of resistance in most community- and hospital-associated gram-negative pathogens make it beneficial for empirical management when rapid control of a serious infection is required (Jana & Deb, 2006; Shakil et al., 2008).

Table 2.3: Types of aminoglycosides (Begg & Barclay, 1995)

Types of Aminoglycosides	**Year of discovery**	**Origin**
Streptomycin	1944	*Streptomyces griseus*
Neomycin	1949	*Streptomyces fradiae*
Kanamycin	1957	*Streptomyces kanamyceticus*
Gentamicin	1963	Actinomycete *Micromonospora purpurea*
Tobramycin	1967	*Streptomyces tenebrarius*
Amikacin	1972	Semisynthetic derivative of kanamycin
Netilmicin	1976	Semisynthetic derivative of sisomisin from micromonospora sp.

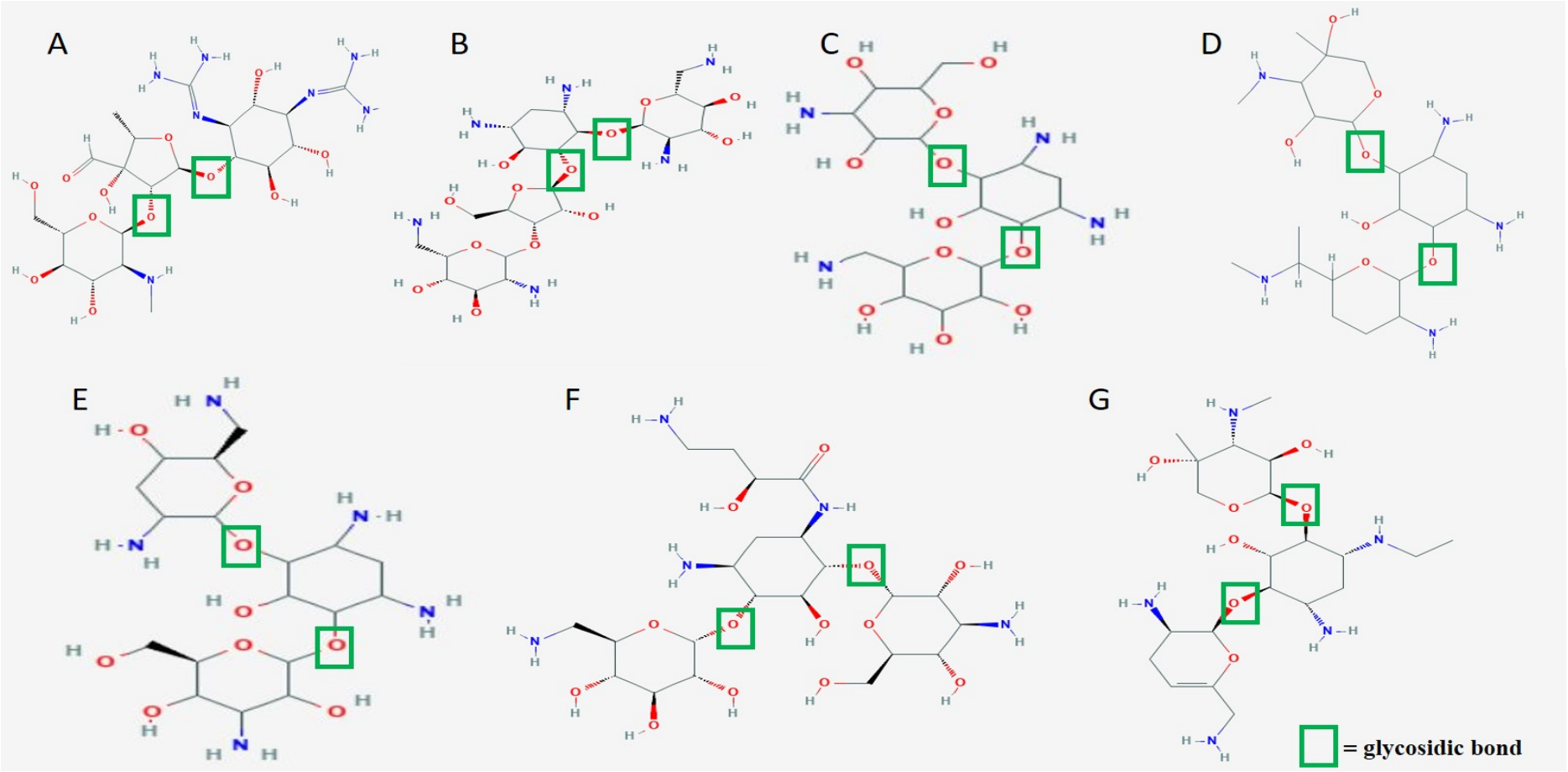

Figure 2.3: Chemical structure of Aminoglycosides antibiotics. A streptomycin , B neomycin, C kanamycin D, Gentamicin, E Tobramycin, F Amikacin, G Netilmicin

2.2.1.1 Mechanism of Action

The basic mechanism of gentamicin in the destruction of bacteria is by impairing bacterial protein synthesis through binding to prokaryotic ribosomes. Gentamicin is diffused through the outer membrane of gram-negative bacteria through disruptions of Mg^{2+} bridges between adjacent lipopolysaccharide molecules (Barclay et al., 1999; Jana & Deb, 2006). Gentamicin is transported into cytoplasmic by active transportation since the size of the gentamicin particle is too large for channel transportation. However, this step is rate-limiting and can be blocked by calcium and magnesium ions, hyperosmolarity, low pH and anaerobic conditions (Barclay et al., 1999; Begg & Barclay, 1995). Through energy dependent transportation, gentamicin binds to the 30S subunit of ribosomes in cytosol and disturbs the elongation of the chain by impairing the reading process which controls translational accuracy (Tulkens et al., 1999). Moreover, gentamicin causes a decrease in translational accuracy and inhibits translocation of the ribosome of a bacterial cell (Yoshizawa et al., 1998).

2.2.1.2 Clinical Use

As one of the most commonly prescribed aminoglycosides, gentamicin can be used alone or in combination with other antibiotics for preventive, empirical or directed therapy. In preventive therapy, only a single dose is sufficient generally before surgical procedures. For empirical therapy, the regime should not exceed 48 hours, and the monitoring of plasma concentration is not required. However, in the case of directed therapy with gentamicin, monitoring of the plasma concentration is

necessary if the treatment is prolonged for more than 48 hours (Avent et al., 2011; Moulds & Jeyasingham, 2010). Directed therapy is limited for specific indications as listed in Table 2.4 (*National Antibiotic Guidelines 2014*, 2014). The extended interval gentamicin doses or once daily dose have shown more benefits and are preferable to traditional multiple daily dosages (Abdel-Bari et al., 2011; Barclay et al., 1999; Lacy et al., 1998). A once daily dosage of gentamicin provides a higher concentration in the blood and reduces the toxicity effects due to a 'gentamicin free' period created during the interval time of administration (Abdel-Bari et al., 2011; Barclay et al., 1999; Lacy et al., 1998; Nicolau et al., 1995).

Table 2.4: Indications for parenteral gentamicin

Preventive therapy **Single dose only** **Alone or combination**	**Empirical therapy** **Duration < 48 hours** **combination**	**Directed therapy** **Duration > 48 hours** **combination**
• Surgical Procedure ❖ Hysterectomy/ laparoscopy ❖ Orthopaedic surgery ❖ Urology surgery ❖ Cardiac surgery ❖ Appendicitis surgery	• Infective endocarditis • Chorioammionitis	• Soft tissues infection • Muscular, skeletal & soft tissue trauma • Compound trauma • Urology infection • Kidney abscess • Acute prostatitis • Acute uncomplicated pyelonephritis

2.2.1.3 Pharmacokinetics and Pharmacodynamics

Gentamicin is poorly absorbed through oral adminstration and it has a half life of 30-60 minutes post administration via intramuscular and intravenous means. It is a non-protein bound drug with a volume distribution (Vd) approximating the volume of

extracellular fluid of 20-35% of body weight (Niemiec et al., 1987; Triginer et al., 1990). However, Vd will expand when there is an increase in volume of extracellular fluid as in patients with ascites, edema, or other enlarged third space (Robertson, 2005). Once in the systemic circulation, their poor lipid solubility prevent the gentamicin being absorbed into most tissues and the majority of the drug pass through glomerular filtration and is renally excreated unchanged form. However a significant propotion typically 15% accumulate within the proximal tubule epitelial cells. A careful dose adjustment of gentamicin is therefore essential for patients with existing renal problems as it is excreted completely by glomerular filtration (Roberts et al., 2012). Gentamicin has concentration-dependent antibacterial activity which means, that the rate of killing the bacteria increases with the increased of gentamicin concentration (Lacy et al., 1998). Studies have shown that, with a higher ratio of gentamicin concentration to minimal inhibitory concentration (MIC) of 8-10:1, the bactericidal effects of gentamicin will be maximised (Moore et al., 1984a, 1984b; Zelenitsky et al., 2003). On the downside, gentamicin has a narrow therapeutic index, which can lead to toxicity if the drug is prescribed without proper monitoring.

2.2.1.4 Nephrotoxicity

Even though gentamicin is well known for its very effective antibiotic properties and low resistance, its usage is limited because of the risk of nephrotoxicity. The clinical manifestation of gentamicin induced nephrotoxicity is nonoliguric renal failure, with a slow rise in serum creatinine and hypoosmolar urinary output, which develop after several days of treatment (Tulkens et al., 1999). It is well known that aminoglycosides are excreted unchanged by glomerular filtration and reabsorbed into

the proximal tubule via endocytosis mechanism mediated by megalin, a 600 kDa multiligand receptor located on apical brush border of the proximal tubule (Choudhury & Ahmed, 2006; Tulkens et al., 1999; Zhai et al., 2000). Megalin, previously known as the Heymann nephritis autoantigen, is a large glycoprotein 330 receptor and member of the low density lipoprotein (LDL) receptor gene family. It is is located mainly in the renal proximal tubule epithelium and labyrinth epithelium of the ears (Farquhar et al., 1995; Zhai et al., 2000) as well as on the retinal epithelium and yolk sac (Zhai et al., 2000). There, gentamicin accumulates within the lysosomes compartment to inhibit lysosomal enzymes, resulting in impaired function and acute tubular necrosis (Radigan et al., 2010). The histopathology of the kidneys has shown severe nephropathy mainly in proximal convoluted tubules nine (9) days after the patients were put on gentamicin treatment (Lenz et al., 2005).

2.3 Current Practice in Diagnosis and Management of Nephrotoxicity caused by Gentamicin

2.3.1 Current Practice to Detect Nephrotoxicity caused by Gentamicin

In ancient Greek medical history, AKI was diagnosed, once there was evidence of a reduction in urine volume (Waikar et al., 2008). As time passed, numerous studies have been conducted worldwide to improve the tools used in the diagnosis and management of AKI. The current indicator used in clinical settings to diagnose AKI is the glomerular filtration rate (GFR), supported by a comprehensive patient history, and physical examination. Patient data, such as vital signs, input/output chart, daily weight, past and current laboratory data (example, serum creatinine (SCr) and blood urea nitrogen), fluid balance and medications are a crucial and classic approach in

helping the diagnosis of AKI and its causes. Data gathered from the patient will be used to diagnose AKI based on the presence of increased serum creatinine and/or blood urea nitrogen (BUN) levels and/or a decreased urine output (Akcay et al., 2010). SCr and BUN have been widely used as biomarkers to diagnose AKI because both are the major by-products of protein metabolism and are normally filtered by the glomerulus. Therefore, any increase in serum concentrations of creatinine and BUN is an indicator of decreased GFR. The baseline renal function can also be estimated using the Modification of Diet in Renal Disease (MDRD) formula or the Cockcroft-Gault formula in adults and the Schwartz formula for children (Table 2.5) (Naughton, 2008; Palevsky et al., 2013). These formulae are useful in categorising the type of AKI for dosage adjustment of drugs when the creatinine clearance (CrCl) falls below 50 ml/min (Naughton, 2008; Palevsky et al., 2013). Hence, the most up-to-date development of tools to diagnose and classify AKI are based on creatinine increase and decrease in the estimated glomerular filtration rate (GFR) or urine output. These are used as biomarkers and are being utilised in the RIFLE criteria (R-renal risk, I-injury, F-failure, L-loss of kidney function, E-end stage kidney disease [ESKD]) and AKIN (Acute Kidney Injury Network) criteria to differentiate the AKI. On the other hand, the detection of nephrotoxicity induced by gentamicin, apart from the above method, comes from therapeutic drug monitoring, linear regression analysis, using population methods or Bayesian estimation procedures (Avent et al., 2011). All the procedures listed can detect nephrotoxicity induced by gentamicin only when there is clear evidence of renal damage. Furthermore, a case reported by Bennett (Bennett et al., 1979) and Edwards (Edwards et al., 1976) have shown that therapeutic drug monitoring of aminoglycosides was not accurate in estimating the

amount of aminoglycosides in the body as a certain level of gentamicin is still detected in the kidney and urine but not in the blood serum despite the discontinuation of gentamicin (Edwards et al., 1976; Gilbert et al., 1979). This is because, half –life of gentamicinin in kidney tissue is 109 hours as compared with serum half-life of 35 minutes (Gilbert et al., 1979). The prolonged accumulation of gentamicin in the kidney might increased the risk for nephrotoxicity.

2.3.2 Current Management of Gentamicin Induced Nephrotoxicity

The management and treatment plans of patients with AKI are varied and depend on etiologic factors. Generally, patients at high risk of developing AKI, such as elderly patients with concomitant chronic diseases, should be monitored closely for AKI. Specifically, guarding against gentamicin induced nephrotoxicity would require the discontinuation of gentamicin after the assessment of benefit-risk of the drug. In addition, laboratory parameters should be obtained vigilantly together with maintenance of optimum hemodynamics, and close surveillance for complications of renal dysfunction (eg: acidosis, electrolyte abnormalities) and controlling sepsis (Akcay et al., 2010; Guo & Nzerue, 2002; Kellum & Lameire, 2013; Lattanzio & Kopyt, 2009).

Table 2.5: Overview table of equation to estimate kidney function

Formula	Estimation Formula	Rationale
CKD-epi eGFR	141 x min $(SCr/\kappa,1)^{\alpha}$ x max $(SCr/\kappa,1)^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if black] κ= 0.7 (females) & 0.9 (males) α= -0.329 (females) & -0.411 [males] min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1.	
MDRD eGFR(Stevens, Coresh, Greene, & Levey, 2006)	eGFR = 175x standardised Scr $(mg/dl)^{-1.154}$ x age (years) $^{-0.203}$ x (0.742 if patient is female) x (1.210 if patient is black) Where GFR is expressed as ml/min/1.73m^2 of body surface area.	To assess renal function stage chronic kidney disease (Levey et al., 1999; Levey et al., 2003)
Cockroft and Gault Creatinine Clearance(Cockcroft & Gault, 1976)	eCrCl = ([140- age (years)] x body weight [kg]) / Scr [µmol/L] x constant Where the constant is 1.23 in male and 1.04 in females. Expressed in ml/min.	To adjust drug dosage for renal function in adults
Schwartz (Schwartz, Haycock, Edelmann, & Spitzer, 1976)	eCrCl = (length [cm] x k) / serum creatinine (mg/dL) k = 0.45: infants 1 to 52 weeks of age 0.55: children one to 13 years of age 0.70: males 14 to 17 years of age 0.55: females 14 to 17 years of age	To adjust drug dosage for renal function in children

eCrCl = estimated creatinine clearance; eGFR = estimated glomerular filtration rate; MDRD = Modification of diet in Renal Disease

2.3.3 Update on Kidney Injury Biomarkers

New markers can now detect kidney injury prior to the rise of SCr and clear evidence of kidney damage. SCr and BUN are well known markers used in the detection of AKI but these biomarkers are considered poor markers of renal dysfunction because SCr and BUN are insensitive and not specific. Scr concentration is greatly influenced by abundant non-renal features (such as body weight, race, age, gender, total body volume, drugs, muscle metabolism, and protein intake) (Coca et al., 2008; Group., 2012; Khwaja, 2012a). Furthermore, acute changes in SCr behind the stage of kidney damage may be delayed; hence using Scr alone to estimate kidney dysfunction may lead to undermining the early detection of kidney injury, thus fail in early diagnosis of sepsis-induced AKI, all of which could also result in further incorrect assessment of treatment efficacy (Coca et al., 2008; Han et al., 2008; Khwaja, 2012b). Therefore, the need for more sensitive and specific biomarkers arises with more research to explore new biomarkers to diagnose AKI earlier. A good diagnostic and monitoring marker should be affordable, accurate, stable, specific and sensitive to the disease/condition under investigation. Other than that, the characteristics needed to formulate biomarkers for acute kidney injury (AKI) are listed in Table 2.6. Several new biomarkers have been proposed and are in various stages of development and validation. Predictor of AKI as listed in Table 2.7 consist of 21 unique biomarkers in both serum and urine from 31 studies (Coca et al., 2008). The biomarkers might be characterized according to their specific performance, for instance: differential diagnosis in established AKI, early detection of AKI and prognosis of AKI.

Table 2.6: Characteristics of ideal biomarker for acute kidney injury

- Non-invasive
- Easily detectable in accessible samples like serum or urine
- Highly sensitive and specific for AKI, also in the presence of concomitant injury involving other organs
- Rapidly and reliably measurable
- Capable of early detection of AKI
- Able to give insight into etiology, nature and duration of insult
- A marker of injury in addition to marker of function
- Predictor of AKI severity and reversibility
- Helpful in monitoring course of and response to interventions
- Useful as surrogate end-point for clinical interventional studies
- Unaffected by other biological variables
- Inexpensive

Table 2.7: New biomarkers to predict AKI

Differential diagnosis in established AKI	**Early detection**	**Prognosis**
• **Serum** ✓ Cystatin C ✓ Carb Hb ✓ NGAL • **Urine** ✓ NGAL ✓ GST ✓ NAG ✓ IL-18 ✓ α-1 microglubolin ✓ NHE3 ✓ KIM-1 ✓ MMP-9	• **Serum** ✓ Cystatin C ✓ Pro-ANP ✓ NGAL ✓ Neutrophil-CD11b • **Urine** ✓ NGAL ✓ IL-18 ✓ KIM-1 ✓ GST ✓ ɤ-GST ✓ π-GST ✓ α-GST ✓ AP ✓ NAG ✓ LDH ✓ MMP-9	• **Serum** ✓ *RRT*: Cystatin C, NGAL ✓ *Death*: IL-6, IL-8, IL-10 • **Urine** ✓ *RRT*: NGAL, Cystatin C, α-1 microglubolin, RBP, β-1 microglubolin, NAG, α-GST, GGT, LDH, KIM-1 ✓ *Death*: NGAL, IL-18, NAG, KIM-1

2.3.3.1 Serum Biomarkers

The most widely discussed biomarkers are cystatin C, which has proven to be a good marker in the detection of AKI at 24 and 48 hours. It has also performed extremely well in identifying established AKI as a marker of glomerular filtration and for predicting the prognosis for RRT. On the other hand, neutrophil gelatinase-associated lipocalin (NGAL) is a good marker for renal tubular inflammation but its sensitivity towards early diagnosis of AKI is low; neither is it able to discriminate between subjects who do not require RRT or who subsequently require RRT (Coca et al., 2008).

2.3.3.2 Urine Biomarkers

The elevation of urinary IL-18, kidney injury molecule-1 (KIM-1) and N-acetyl-β-D-glucosaminidase (NAG) are also predictors of AKI caused by acute tubular necrosis. The same study revealed that, the combinination of KIM-1, NAG and metalloproteinase-9 (MMP-9) yielded perfect results for diagnosing AKI. Sodium-hydrogen exchanger form-3 (NHE-3) is also a good marker in detecting proximal tubule injury. Urinary IL-18 and urinary NGAL are good metabolites as an early predictive biomarker of AKI; even though urinary IL-18 has low sensitivity, it has a high specificity and rarely shows false positive elevation in AKI. In the case of risk-stratified patients who are undergoing critical dialysis, urinary KIM-1, NAG and IL-18 are the most beneficial markers compared with the others. There are also studies showing metabolite changes in the urine when induced by gentamicin in rats. The metabolites identified are serine, threonine, glutamine, agmatine and phosphate while

5-methyltetrahydrofolate is a putative biomarker in the kidney tissue for nephrotoxicity induced by gentamicin (Boudonck, Mitchell, et al., 2009; Boudonck, Rose, et al., 2009).

2.4 The Potential Role of Pharmacometabonomics in Nephrotoxicity

Pharmacometabonomics is a new method arising from -omics and has a very promising potential in detecting toxicity. As defined by Clayton et el, pharmacometabonomics is the prediction of the outcome of a drug or xenobiotic intervention in an individual based on mathematical models with a pre-intervention metabolites signature (Clayton et al., 2006). The advantages of these new methods are the ability to personalise drug treatments according to each patient's needs; this can therefore maximise treatment benefits and minimise side effects. Ideally, each patient will have the right medicine at the right dosage at the right time. With this new technology, urine was used to predict the effectiveness of the treatment, which is more convenient and non-invasive for the monitoring of drugs. Previous studies have shown the application of pharmacometabonomics as a predictor of toxicity in drug management (Table 1.8).

Therefore, aimed of this study, to investigate the ability of the pharmacometabonomics technique to predict aminoglycoside induced nephrotoxicity in human. Gentamicin will be used because it is commonly prescribed and the most toxic of aminoglycosides compared with others (Paul, 1986). As more people are now looking more into personalised medicine or treatment, scientists globally are endeavouring to identify the complexities of personalised medicine.